

Volatile Compounds Produced by Copper-Catalyzed Oxidation of Butterfat

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Abstract

Anhydrous butterfat treated with copper and stored under vacuum at 38C in the dark for 9 weeks yielded the C₅₋₈ *n*-alkanes, acetic acid and the C_{4,5,7,9} alkan-2-ones. The same butterfat stored under oxygen for 1 week yielded the C_{1,2,3,5-7} *n*-alkanes, the C_{2,4,6,8} alk-1-enes, the C_{7,8} alk-1-ynes, the C₂₋₆ *n*-alkanoic acids, ethanol, the C₂₋₇ *n*-alkanals, 3-methylbutanal and 4-methylpentanal, the C₁₋₄ *n*-alkyl formates, ethyl acetate and propionate, *n*-propyl acetate and propionate, and butanone. Both treatments yielded carbon dioxide. All samples including those not treated with copper yielded butane-2,3-dione.

Introduction

CHEMICAL MECHANISMS for the formation of hydroperoxides in oxidizing lipids predict the presence of a wide range of compounds. During the last 15 years an enormous amount of time has been spent identifying the volatile compounds from oxidizing lipids, and investigators generally attributed the characteristic flavors to mixtures of *n*-alkanals, *n*-alk-2-enals and *n*-alka-2,4-dienals. Much of this information was obtained through derivatives, in particular, 2,4-dinitrophenylhydrazones.

More recently, single compounds have been identified and claimed to be responsible for characteristic lipid flavors, e.g., green [hex-*cis*-3-enal (6)], creamy [hept-*cis*-4-enal (5)], metallic [oct-1-en-3-one (17)] and mushroomy [oct-1-en-3-ol (7,18)].

In the last few years modern analytical techniques have made possible the comprehensive and quantitative study of these volatile compounds. For example, Stark and Forss (19) have identified the C_{1,2,5-8} *n*-alkan-1-ols in butter prepared from oxidized cream and compared the amounts with those of the *n*-alkanals and *n*-alk-2-enals also present.

Employing chromatography and spectrophotometry, Kawahara et al. (11) and Kawahara and Dutton (10) identified several aldehydes from autoxidized soybean oil and pure methyl linoleate, and attributed the painty and rancid odors to these and possibly other carbonyl compounds. In another study, employing combined gas chromatography and mass spectrometry, Horvat et al. (8) identified the volatile compounds from methyl linoleate treated with oxygen in diffuse daylight. The principal components were *n*-pentanal, *n*-hexanal, amyl formate, methyl octanoate, and substituted dioxolanes. Minor components included esters, alcohols, ketones, aldehydes, hydrocarbons, and acetals.

The aim of this study was to conduct a carefully controlled experiment to determine which volatile compounds were produced by copper-catalyzed oxidation of anhydrous butterfat stored at 38C in the dark, both with and without oxygen. The amounts of all the volatile compounds were determined; the

use of an all-glass system and the avoidance of solvents minimized the presence of artifacts.

Experimental

A 50-pound can of butterfat was obtained from a commercial source. The fat was melted at 45C and put into No. 2-C-enameled tin cans which were sealed under vacuum and stored at -18C. When needed, the butterfat was melted in the can at 45C and 200 g poured into a 1-liter round bottom flask with a glass encased magnetic stirring bar. The butterfat was solidified in an ice bath and a break-seal assembly attached as described in the preceding paper (1).

Four 200 g samples were used as controls and four for experiments. The butterfat at 45C was degassed through the side arm and held at 10⁻³ torr for 6 hr with continuous stirring. For controls two of the flasks were flame-sealed under high vacuum while two were sealed under one atmosphere of oxygen admitted from a flexible polyethylene bag through the side arm. Gas chromatograms were obtained of the samples of the oxygen to ensure that contaminants were not added with the oxygen. The experimental samples were prepared for oxidation in the same way except that one part per million of copper as cupric stearate was added to the butterfat before the break-seal assembly was attached. The eight samples were stored in the dark at 38C.

Four samples, one of each of the four treatments, were analyzed for volatiles after one day's storage. Analyses of volatiles in the remaining four samples were carried out after a 1 and 9 weeks' storage. At the specified time the reaction flask was attached to a high vacuum system and headspace and total condensate samples obtained. The total condensate was collected for 8 hr with continuous pumping (10⁻³ torr) and stirring of the fat held at 45C. Aliquots of the headspace and the total condensate were analyzed by gas chromatography and mass spectrometry (1). 2,4-Dinitrophenylhydrazones were analyzed by thin-layer chromatography (20).

Results

The fresh butter odor present in the controls was replaced by a characteristic oxidized odor in the samples treated with copper but only the samples treated with copper and stored under oxygen showed change in color. After 1 week's storage the butterfat was completely bleached.

No volatile compounds (except for a trace amount of butane-2,3-dione) were found in any of the controls. Many volatile compounds were collected from the copper-treated butterfat. These are listed in Table I. There is a small number of major components and a large number of minor ones. Fewer volatiles were found in the evacuated than in the oxygen-containing samples.

n-Alkanals predominated in the samples treated with copper and stored under oxygen. This was more evident in the oxidized sample stored for one week

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T —	Volatile Trace	Compounds from S — Small	Copper-oxidized M — Moderate	Butterfat L — Large	
		Stored under vacuum		Stored under oxygen	
		1 day	9 weeks	1 day	1 week
Alkanes					
Methane				S	S
Ethane				S	S
Propane				S	S
<i>n</i> -Pentane		T	M		M
<i>n</i> -Hexane		T	M	M	M
<i>n</i> -Heptane			M	M	S
<i>n</i> -Octane			M	M	
Alkenes					
Ethene					S
But-1-ene				S	S
Pent-1-ene				M	M
Hex-1-ene					M
Oct-1-ene					M
Alkynes					
Hept-1-yne					S
Oct-1-yne					S
Acids					
Acetic			S	S	L
Propionic				S	S
<i>n</i> -Butyric				S	S
<i>n</i> -Pentanoic				S	S
<i>n</i> -Hexanoic				S	S
Alcohols					
Ethanol					S
Aldehydes					
Ethanal				T	S
Propanal				M	L
<i>n</i> -Butanal				S	M
<i>n</i> -Pentanal				S	L
<i>n</i> -Hexanal					L
<i>n</i> -Heptanal				S	L
3-Methylbutanal					S
4-Methylpentanal					S
Esters					
Methyl formate				S	S
Ethyl formate				S	M
<i>n</i> -Propyl formate					S
<i>n</i> -Butyl formate				S	S
Ethyl acetate					S
<i>n</i> -Propyl acetate					S
Ethyl Propionate					S
<i>n</i> -Propyl Propionate					S
Ketones					
Butanone			T	T	
Pentan-2-one			M		
Heptan-2-one			L		
Nonan-2-one			M		
Others					
CO ₂		S	S	S	S
O ₂				L	L

than in the one stored for one day. The C_{3,5-7} *n*-alkanals were produced in largest quantities (see Table II). *n*-Butanal was produced in moderate amount, and ethanal, 3-methylbutanal and 4-methylpentanal were found in small amounts. No aldehydes containing more than 7 carbons were found.

A larger number of hydrocarbons was produced than any other class of compounds but none occurred in large quantity. The C_{5,6} *n*-alkanes were found in moderate amounts while the C_{1-3,7} *n*-alkanes were found in small amounts. The C_{5,6,8} *n*-alk-1-enes were present in moderate amounts while the C_{2,4} *n*-alk-1-enes were present in small amounts. Hept-1-yne and oct-1-yne were present in small amounts. Hydrocarbons present after one day's storage did not appear to increase in amounts with longer storage, but several

hydrocarbons not found after one day's storage were found after one week's storage. The apparent decrease of heptane and octane after one week's storage is most likely due to incomplete chromatographic separation of these alkanes from large amounts of pentanal and hexanal.

Large amounts of acetic acid, moderate amounts of ethyl formate and small amounts of other esters and acids were also found. Ethanol and butanone were also detected in small amounts. A small amount of carbon dioxide was found in all samples and a large amount of oxygen remained in those samples to which it was added.

Although fewer compounds were produced in the samples treated with copper and stored under vacuum, the majority of those found after 9 weeks' storage were present in moderate to large quantities. Heptan-2-one was produced in large quantity, pentan-2-one, nonan-2-one, *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane in moderate quantities, and butanone and acetic acid in small quantities. The evacuated samples stored for one day contained only small amounts of *n*-pentane, *n*-hexane and carbon dioxide.

Discussion

Oxidation of fats generally proceeds via a free radical mechanism which may be initiated by a metal ion (in this case Cu⁺⁺) or by a radical with removal of hydrogen from a methylene group α to a double bond of the fatty acid chain (9). Reaction of the radical with oxygen produces hydroperoxides which on decomposition can produce aldehydes and ketones. Polymerization or disproportionation with other radicals may then lead to the formation of further aldehydes, hydrocarbons, alcohols, acids and esters.

The carbonyl compounds found in this study are fewer in number than those found by other investigators (3,4) which could be due to the method employed to oxidize the fat, the low level of water and the length of time allowed for reaction. Pentanal, hexanal, and heptanal, all present in oxidized butterfat in large amounts, could be derived from arachidonic (0.2% in butterfat) and linoleic (3%) acids. Propanal, also found in relatively large amounts, could be formed by oxidation of dodec-9-enoic, but mainly from linolenic acid (1%) since its production as well as that of butanal was rapid after only a one-day storage period.

The fact that propanal, probably derived from linolenate, was present after one day and hexanal, probably derived from linoleate, only appeared after one week of storage, and the fact that none of the aldehydes expected to be derived from oleate were found even after one week is consistent with the reactivity of these unsaturated esters (11). The absence of unsaturated aldehydes in the oxidized samples supports previous evidence (12) that the methylene group α to the double bond most removed from the ester group in the fatty acid chain is preferentially attacked, or that the unsaturated aldehydes were themselves oxidized (14).

The appreciable amounts of pentan-2-one, heptan-2-one and nonan-2-one in samples stored under vacuum cannot be explained, but these methyl ketones can be formed from 3-keto acids in butterfat heated in the presence of traces of water, e.g. at 140°C for 3 hr (13). Heptan-2-one was identified by Forss et al. (4) in butterfat oxidized with a mixture of

TABLE II
Estimated Amounts of *n*-Alkanes and *n*-Alkanals in
Copper Catalyzed Oxidized^a Butterfat

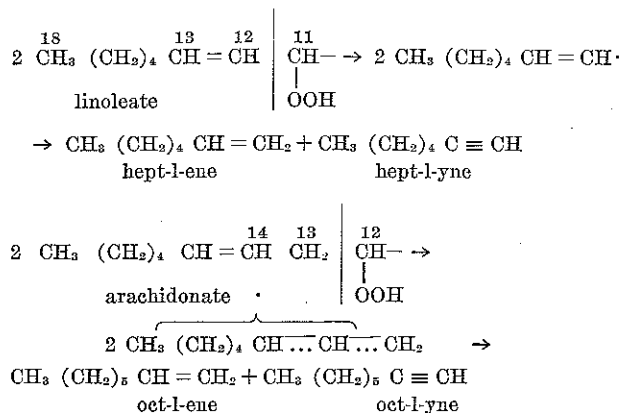
Compound	$\mu\text{M}^b/\text{kg}$
Alkanes	
Methane	0.5
Ethane	0.5
Propane	0.5
Pentane	3
Hexane	2
Heptane	0.5
Alkanals	
Propanal	10
Butanal	3
Pentanal	15
Hexanal	35
Heptanal	30

^a Stored 1 wk under 1 atm O₂ at 88°C.

^b Estimated from thin-layer and gas chromatography data.

The presence of hydrocarbons in the copper-treated butterfat probably results from reactions of alkyl free radicals with each other or by abstraction of a hydrogen atom from a fatty acid chain. Buttery (2) has isolated hydrocarbons from oxidized potato granules. The presence of *n*-pentane, *n*-hexane, *n*-heptane and *n*-octane in butterfat with copper stored in a vacuum, but not in butterfat without copper stored under vacuum, indicates that the copper may have catalyzed the decomposition of lipid hydroperoxides already present in the butterfat before treatment or that copper itself was able to form sufficient free radicals from the lipids during the 9-weeks' storage.

Hept-1-yne and oct-1-yne were probably derived from the 11-hydroperoxide of linoleate and the 13-hydroperoxide of arachidonate, and the 12-hydroperoxide of arachidonate, respectively, by formation of the alk-1-ene free radical and its disproportionation to the acetylenic and vinyl compounds (16). For example, see scheme I.



SCHEME I

Free radical formation does not require oxygen. The presence of a limited number of hydrocarbons found in the sample stored under vacuum suggests that copper may initiate free radicals directly in the vicinity of double bonds in the fatty acid chains. The direct formation of free radicals in the alkyl chain of the fatty acid plays a great part in the formation of volatile compounds in irradiated fats. The next paper describes the identification of volatile compounds from irradiated butterfat.

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